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Heterogeneity of human eosinophil glucocorticoid receptor expression in hypereosinophilic patients: absence of detectable receptor correlates with resistance to corticotherapy

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SUMMARY

Assessment of steroid receptor content in human neoplastic lymphoid cells or mammary tumour cells has been previously used to predict steroid sensitivity in various types of cancers. In the present study, we have evaluated the relationship between glucocorticoid receptor content and the glucocorticoid sensitivity of human eosinophils, since hypereosinophilic patients do not always respond favourably to glucocorticoid, particularly in the hypereosinophilic syndrome (HES). Blood or alveolar eosinophils obtained from seven patients (four with HES without leukaemic markers; two with parasitic diseases; and one with eosinophilic pneumonia) displayed the same specific glucocorticoid receptor content as normal eosinophils ($7{\cdot}58 \pm 1.31 \times 10^3$ *versus* $7{\cdot}76 \pm 0{\cdot}74 \times 10^3$ sites/cell). In contrast, glucocorticoid-binding sites were undetectable in purified eosinophils collected from seven HES patients with ($n=3$) or without ($n=4$) leukaemic markers, whilst their mononuclear cells and/or neutrophils bound glucocorticoid. In one HES patient, kinetic studies showed that blood eosinophils initially positive in glucocorticoid binding assays became negative with the subsequent appearance of leukaemic markers. The absence of specific glucocorticoid binding sites was correlated with the absence of glucocorticoid receptor proteins by the use of a specific anti-glucocorticoid receptor monoclonal antibody. Eosinophil sensitivity to glucocorticoid was investigated by the evaluation of glucocorticoid inhibition of eosinophil chemotaxis and by the clinical outcome of *in vivo* glucocorticoid therapy. Our data provide evidence of the heterogeneity of eosinophil glucocorticoid receptor expression. In addition, the presence of glucocorticoid receptors is a prerequisite for glucocorticoid activity, *in vitro* and *in vivo*, on cells of the eosinophil lineage.

Keywords eosinophils glucocorticoids hypereosinophilic syndrome

INTRODUCTION

Hypereosinophilic syndrome (HES) is defined as a blood hypereosinophilia $> 1{\cdot}5 \times 10^9$/l for at least 6 months and leading to possible multi-organ system lesions (e.g. cardiopathy, neuropathy), and includes a variety of distinct diseases of unknown pathogenesis. An underlying malignant process has been suggested in some HES by the presence of leukaemic markers (Flaum *et al.*, 1981) and the possible evolution into a leukaemia or a T cell lymphoma (Schooley *et al.*, 1981; O'Shea *et al.*, 1987; Prin *et al.*, 1988). While therapeutic doses of glucocorticoids commonly induce peripheral blood eosinopenia (Kellgren & Janus, 1951), this eosinopenic effect is quite variable with respect to the disease entities associated with blood or tissue hypereosinophilia. The ability to respond to glucocorticoid therapy appears to be a major criterion for discrimination between benign and grave forms of HES (Chusid *et al.*, 1975; Bush *et al.*, 1978) and glucocorticoid resistance has been noted in malignant HES (Parillo, Fauci & Wolff, 1978; Schooley *et al.*, 1981).

We investigated the presence of glucocorticoid binding sites on highly purified eosinophils freshly obtained from healthy donors or distinct hypereosinophilic patients with various etiologies. In particular, we studied HES patients having leukaemic markers (increased serum vitamin B12, abnormal leucocyte alkaline phosphatase scores) or elevated serum IgE levels, with or without multi-organ dysfunction (endomyocardial fibrosis, neuropathy). As previously shown (Peterson *et al.*, 1981), normal blood eosinophils possess high-affinity glucocorticoid binding sites. In contrast, blood eosinophils from hypereosinophilic patients appear quite heterogeneous in their ability to bind and respond to glucocorticoid. The significance of such an eosinophil glucocorticoid receptor defect in relation to the clinical severity of the HES is discussed.

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Table 1. Source of human eosinophils with glucocorticoid binding sites

Patient no.	Sex	Blood eosinophil count %	Blood eosinophil count nb × 10^9/l	Clinical diagnosis (Main clinical or biological signs)	Tested cells* Band	Tested cells* %	^{3}H-dexamethasone Binding (sites/cell)
1	M	3	0·174	Healthy	V	96 (Eo)	7020
2	F	4	0·380	Healthy	IV	97 (Eo)	8500
					III	73 (N)	9430
					I	85 (L)	5660
3	M	20	1·120	Filariasis; serum IgE level 1200 KUI/l	IV	95 (Eo)	7300
		18	1·010		IV	96 (Eo)	8200
4	M	17	1·400	Anguillulosis	IV	85 (Eo)	8600
		25	1·750		IV	90 (Eo)	9400
5†	F	13	1·250	Chronic eosinophilicpneumonia;	V	90 (Eo)	8200
		66‡		serum IgE level 840 KUI/l	IV	98 (Eo)	7010

* Highly purified eosinophils (Eo), neutrophils (N) or mononuclear cells (L) collected in metrizamide gradients.
† Patient who received prednisone (60 mg daily) after the study.
‡ Alveolar cell count: total cell number collected after BAL: $24·5 \times 10^7$ with 66% eosinophils.

MATERIALS AND METHODS

Eosinophils

Eosinophils were obtained from two healthy volunteers having normal counts of blood eosinophils (<400/mm^3) and 14 hypereosinophilic patients of various etiologies (Tables 1, 2). Informed consent was obtained from all participants. Except for one case mentioned in Table 2, the eosinophilic patients did not receive any therapy for 3 weeks before the study. The range of plasma cortisol levels, between 11·8 and 20·9 μg/100 ml at 8 AM, was normal. The processing of blood samples was the same for all patients. Blood leucocytes were initially separated from heparinized venous blood by dextran sedimentation and washed in minimal essential medium (MEM, Difco, Detroit, MI). In one patient with eosinophilic lung disease, alveolar cells were recovered by bronchoalveolar lavage (BAL). After filtration of the lavage through several layers of sterile surgical gauze, the cells were separated from the lavage fluid by low-speed centrifugation (800 *g* for 10 min) at 4°C. The pellet was suspended in MEM supplemented with 100 IU Penicillin/ml and 50 μg streptomycin/ml (Specia, Paris, France).

Purification of eosinophils

Blood and alveolar eosinophils were purified by centrifugation on discontinuous metrizamide gradients as previously detailed (Prin *et al.*, 1983; 1986). By using this separation procedure, distinct populations of eosinophils are collected which sediment in fractions of low density (20, 22 and 23% metrizamide solutions; interfaces I, II, III), intermediate density (24% metrizamide solution; interfaces IV) or a high-density zone (25% metrizamide solution; interface V). After three washings in phosphate-buffered saline (PBS) the cells of each interface were resuspended in 5 ml MEM and evaluated for total number and differential cell counts. The degree of eosinophil purity (cytocentrifuge smears and Giemsa staining) was estimated for each band. Only layers containing more than 85% of eosinophils were used. In parallel, purified mononuclear cells (⩾70%; band I, II) and neutrophils (⩾75%; band III) were used in some studies as controls for the binding assays. The viability and vitality of purified eosinophils were assessed, respectively, by the trypan blue dye exclusion technique and by studies of cell ATP content using the luciferin–luciferase assay as previously described (McElroy & Seuger, 1963).

Steroid binding assays

Cell suspensions (3·0–15 × 10^6 cells/ml) were diluted in steroid-free MEM medium (MEM supplemented with 1% glutamine, 2% ultroser; SF, I.B.F., Villeneuve La Garenne, France; and adjusted to pH 7·4 with 20 mM HEPES). One-millilitre samples of cell suspensions from freshly fractionated leucocytes were incubated with various (4–40 nM) concentrations of [6, 7$_n$-^{3}H] Dexamethasone (91 Ci/mmol, NEN, Boston, MA) for 2 h at 37°C. The cells were then washed three times with ice-cold calcium-free HBSS and pelleted. Radioactivity was extracted from cell pellets with 100 μl ethanol and assayed by scintillation counting. Specific binding (Bs) was determined in duplicate by comparing radioactivity in intact cells when incubated with [^{3}H] dexamethasone alone (T) and in the presence of a 100-fold molar excess of the same unlabelled steroid (B). (Bs = T − B). Non-specific binding accounted for 40–50% of total cellular binding. The number of saturable binding sites per cells and the dissociation constant (K_d) were estimated by Scatchard analysis. (Free-labeled dexamethasone was measured in the supernatant of centrifuged cells). In order to remove endogenous bound steroid, freshly isolated leucocytes were incubated in steroid-free medium for 3 h prior to assays.

Immunochemical detection of the glucocorticoid receptor protein

Cytosolic and nuclear extracts were prepared as follows: 1 × 10^8 cells were pelleted by centrifugation (800 *g*, 5 min), washed and resuspended in buffer A (20 mM potasium phosphate, pH 7·4; 130 mM KCl, 1·5 mM $MgCl_2$; 1 mM EDTA; 20 mM β-mercaptoethanol; 10% glycerol; 1 mM phenyl-methyl-sulphonyl fluoride and 1 mg/ml leupeptin) and then homogenized in 0·2 ml of the same buffer using a teflon-glass homogenizer. A low-speed centrifugation (4000 *g* for 10 min at 4°C) was then

performed, with the supernatant representing the cytosol extract. Nuclear receptors were extracted from particulate pellets with buffer A supplemented with 0·4 ml KCl (45 min at 0°C). Bradford's method (Bradford, 1979) using microassay procedure outlined in the Bio-rad technique was employed to estimate the protein concentration in cell extracts. Conventional sodium dodecyl sulphate–polyacrylamide gel (7·5%) electrophoresis (SDS–PAGE), and protein blotting were performed as previously described (Towbin, Stahelin & Gordon, 1979); 10 μg of total protein were loaded in each track. A mouse monoclonal antibody against the rat glucocorticoid receptor (MoAb 7) that cross-reacts with the human glucocorticoid receptor (Brönnegard *et al.*, 1987) was kindly provided by Prof. J. A. Gustafsson (Karolinska Institutet, Huddinge University Hospital, Sweden). Peroxidase-conjugated anti-mouse IgG antibodies (Institut Pasteur, Paris, France) were used for the detection of receptor–MoAb complexes.

Chemotaxis assay

Eosinophil migration was tested in modified Boyden chambers, using micropore filters (Gosset *et al.*, 1986). The assay was carried out in a 48-well microchemotaxis assembly (Neuroprobe, Cabin John, MD). Polycarbonate filters, pore size 5 μm (Nucleopore, Pleasanton, CA) were used to separate the upper and lower compartments of chemotaxis chambers. Cell suspensions containing more than 85% pure eosinophils were placed in the upper compartments. The cells were resuspended in HBSS with 15 mM HEPES and adjusted to 5×10^5 cells/500 μl. In some experiments, eosinophils were treated with either dexamethasone (10^{-6} to 10^{-9} M) or dexamethasone in the presence of a 100-fold excess of RU 486, a glucocorticoid antagonist (Gagne, Pons & Philibert, 1985) (RU 486 was kindly provided by Dr D. Philibert Roussel, Uclaf, Romainville, France). The stimulus (platelet-activating factor (PAF) acether at 10^{-6} M) (a generous gift from Dr P. Braquet, Institut de Recherche Thérapeutique Beaufour, Robinson, France) or control agents (HBSS, dexamethasone, RU 486,) were placed in the lower compartments. Chemotaxis chambers were incubated for 2 h at 37°C in a humid atmosphere of 5% CO_2; the filters then removed, fixed and Giemsa stained. The number of eosinophils that had migrated was determined microscopically using an oil immersed-objective (× 1000). Eosinophils were enumerated in four random high-power fields (HPF) in quadruplicate wells (means ± s.e.m. of 16 measures for each test). Cell counts on test filters were made using a double-blind procedure.

Statistical analysis

Results were expressed as the mean ± s.e.m. and group comparisons were performed using Student's *t*-test.

Table 2. Leucocyte glucocorticoid binding sites in HES patients

Patient no.	Sex	Blood eosinophil count %	Blood eosinophil count nb × 10^9/l	Clinical diagnosis* (Main clinical or biological signs)	Tested cells† Band	Tested cells† %	^{3}H-dexamethasone Binding (sites/cell)
6‡	M	24	2·200	Serum IgE level 2890 KUI/l	IV	87 (Eo)	7200
7	M	44	3·920	Lung involvement	IV	93 (Eo)	2300
8	F	47	4·935	Weight Loss;	IV	89 (Eo)	13850
				serum IgE level 243 KUI/l	II	70 (L)	4340
9	M	59	16·400	Serum IgE level 1588 KUI/l	IV	97 (Eo)	5682
		70	17·220	Elevated vitamin B12 levels	IV	95 (Eo)	0§
10‡	F	74(1)	33·450	Nervous system involvement	III	89 (Eo)	0
		78(2)	49·700		III	92 (Eo)	0
11‡	M	74	33·450	Cardiac involvement; elevated	IV	85 (Eo)	0
				vitamin B12, low LAP score	III	75 (N)	8450
					I	95 (L)	5660
12	M	35	2·730	Serum IgE level 450 KUI/l	IV	85 (Eo)	0
					I	84 (L)	5500
13	F	41	3·200	Anorexia; weight loss;	IV	98 (Eo)	0
				elevated vitamin B12 level;			
				serum IgE level 150 KUI/l	III	85 (N)	6500
14‡	M	45	9·140	Endomyocardial fibrosis;	III	92 (Eo)	0
				elevated vitamin B12 level			
15	F	36	3·100	Skin involvement	IV	92 (Eo)	0
16	M	36	1·720	Endomyocardial fibrosis	IV	88 (Eo)	0

* Patients offering the diagnostic criteria of the hypereosinophilic syndrome (HES) as defined by Chusid *et al.* (1975).

† Highly purified eosinophils (Eo), neutrophils (N) or mononuclear cells (L).

‡ Patients who received prednisone (50 to 60 mg daily) after the study. In one patient (no. 10); the work was performed before (1) and after (2) the administration of steroids.

§ A receptor level 0 represents a number undetectable above background in the assay, i.e. less than 1000 sites/cell.

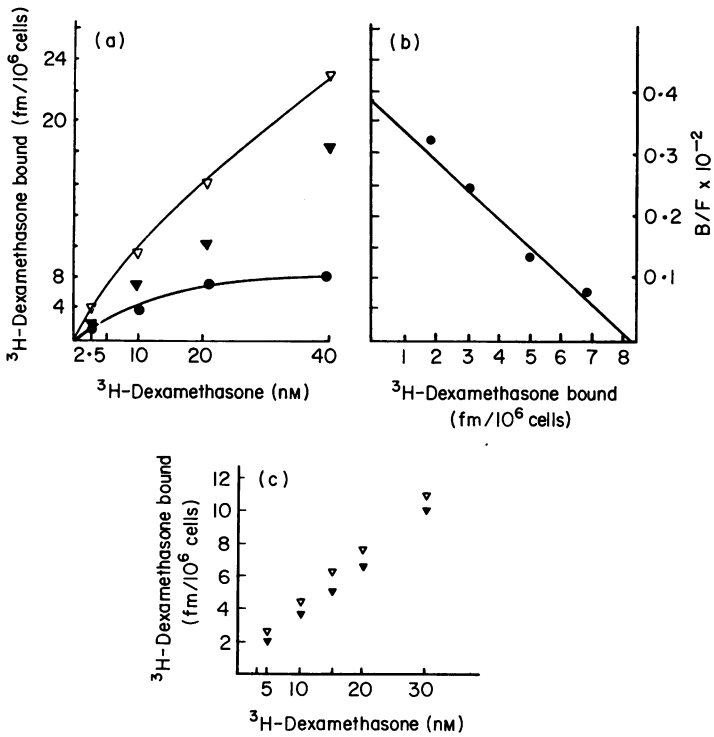

Fig. 1. Representative experiments of ^{3}H-dexamethasone binding (a) to eosinophils from patients having (a, b) or lacking (c) glucocorticoid binding sites. (b) Scatchard analysis of specific binding to eosinophils. ▽, total; ▼, non-specific; and ●, specific binding.

RESULTS

Variable presence of glucocorticoid binding sites in human eosinophils

A whole cell ^{3}H-dexamethasone binding assay was used to determine the relative amount of steroid bound by freshly fractionated eosinophils from two healthy donors and 14 patients with blood or alveolar hypereosinophilia of vaious etiologies (Tables 1, 2). Patients could be separated into two groups based on the number of ^{3}H-dexamethasone binding sites. In a first group of subjects, eosinophils possessed $7{\cdot}58 \pm 1.31 \times 10^3$ dexamethasone binding sites/cell with K_d of $16{\cdot}05 \pm 0{\cdot}21$ nM at 21°C (Fig. 1). This pattern of steroid binding was very similar to that observed with eosinophils from the two healthy donors ($7{\cdot}76 \pm 0{\cdot}74 \times 10^3$ receptor sites per cell with a K_d of $16{\cdot}0 \pm 0{\cdot}2$ nM). In contrast, the eosinophils from a second group of patients exhibited undetectable glucocorticoid receptor levels (Fig. 1). More than 90% of tested eosinophils excluded trypan blue. Eosinophils lacking detectable binding sites ($< 1 \times 10^3$ sites/cell) contained a similar ATP content to cells having binding sites ($1{\cdot}92 \pm 0{\cdot}60$ nmol/10^6 cells, cases 13 and 15 *versus* $1{\cdot}79 \pm 0{\cdot}52$ nmol/10^6 cells, cases 4 and 7). In the two groups of patients, the binding assays were predominantly performed with highly purified eosinophils ($\geqslant 85\%$ pure eosinophils) collected in band IV (13 out of 20 tests) with positive ($n = 7$) or negative binding assays ($n = 6$). The lack of detectable binding sites was not due to an increase in background non-specific binding. The latter was variable and could only be considered for a given patient. Parallel to the defect in eosinophil glucocorticoid binding, control assays on enriched mononuclear cells or neutrophils from the same patients

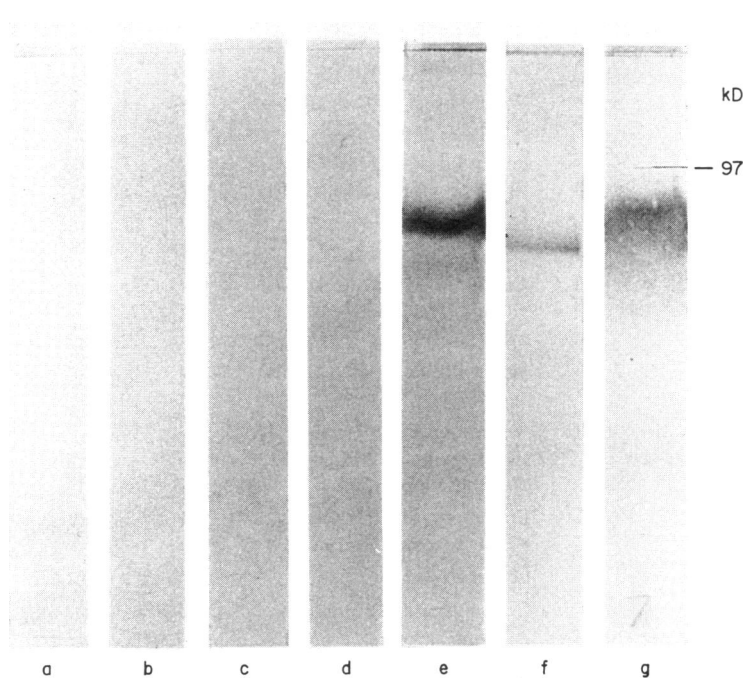

Fig. 2. Immunoblot analysis of the cytosolic and nuclear fractions of eosinophils: eosinophils obtained from subjects negative for specific glucocorticoid binding assays (cases 13 and 15, Table 2) with, respectively, cytosolic (a, b) and nuclear fractions (c, d). Cytosolic fractions of blood (e) or alveolar eosinophils (f) respectively obtained from subjects positive for specific glucocorticoid binding assays (cases 3 and 5, Table 1). Cytosolic preparations of rat thymus extract (g) were used as positive controls. The mouse monoclonal antibody against the glucocorticoid receptor is MoAb 7 as specified in Materials and Methods.

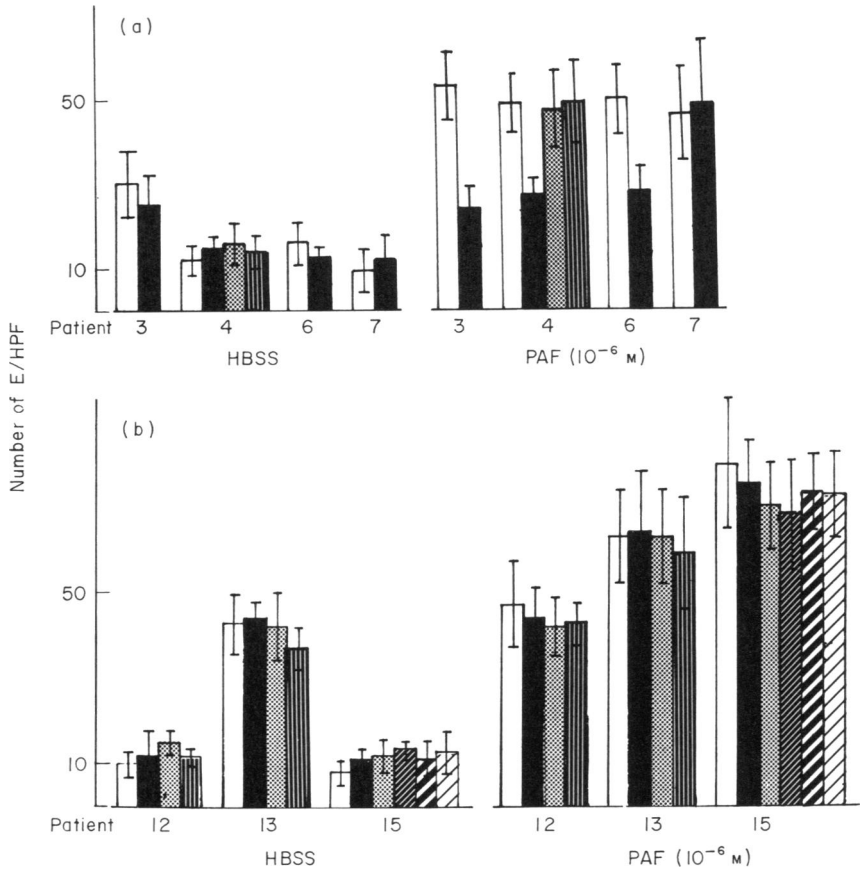

Fig. 3. The migration of eosinophils (E) having (a) or lacking (b) glucocorticoid binding sites, towards HBSS (spontaneous locomotion) or PAF at 10^{-6} M (chemotaxis). (a) The eosinophils were incubated 2 h with either HBSS (□); or with dexamethasone at 4×10^{-6} M (■); dexamethasone in the presence of 100-fold excess of RU 486 (▨) at 10^{-6} M, as described in Materials and Methods. (b) A representative experiment illustrates the dose-dependent effect of dexamethasone (■, 10^{-6} M; ▩, 10^{-7} M; ▨, 10^{-8} M; and ▨, 10^{-9} M) on eosinophil chemotaxis with cells lacking glucocorticoid binding sites. The number of eosinophils/HPF is the mean ± s.e.m. of 16 measurements for each test. All tested eosinophils were collected from band IV.

revealed their ability to bind the steroid (Table 2). The results of the binding assays were shown to be reproducible in successive experiments during the course of the illness (maximal on follow-up of 18 months) and before or after prednisone therapy in steroid-insensitive patients (Tables 1, 2). Nevertheless, the glucocorticoid binding capacity of eosinophils, collected in band IV, was shown to disappear in one HES patient (Table 2; case 9) 11 months after a previous positive binding test.

To define more precisely the origin of the binding defect, comparative immunoblot analyses were performed with pure eosinophils ($\geqslant 90\%$) having ($n=2$) or lacking ($n=2$) glucocorticoid binding sites. As shown in Fig 2, only cytoplasmic preparations from eosinophils having dexamethasone binding sites contained a 90 kD band which appears antigenically related to the glucocorticoid receptor protein detected in rat thymus extract.

Glucocorticoid binding ability related to in vitro *or* in vivo *eosinophil sensitivity to glucocorticoid*

Glucocorticoid may induce blood eosinopenia by inhibiting eosinophil migration (Altman, Hill & Hairfield, 1981). *In vitro* eosinophil sensitivity to glucocorticoid was tested in a chemotaxis assay. As shown in Fig. 3, random eosinophil migration was not notably modified after dexamethasone treatment. In contrast, three out of four assays with eosinophils having glucocorticoid binding capacities exhibited a statistically significant decrease in eosinophil chemotaxis to PAF after *in vitro* incubation with dexamethasone at 4×10^{-8} M ($P < 0{\cdot}05$; Fig. 3a). In addition, the presence of RU 486, an anti-glucocorticoid displaying a high affinity for the glucocorticoid receptor, prevented the inhibitory effect of dexamethasone. This inhibitory effect was probably due to the antiglucocorticoid effect of RU 486, and not to the other properties of this multifaceted compound since DXB, a selective anti-glucocorticoid (Rousseau *et al.*, 1979) had an identical effect in this assay (data not shown). In contrast the inhibitory effect of dexamethasone was never observed in eosinophils lacking glucocorticoid receptors (Fig. 3b).

As mentioned in Tables 1 and 2, prednisone therapy was introduced in five patients. All patients received therapeutic doses of prednisone (1 mg/kg) for 5 days. An almost complete disappearance of circulating eosinophils was observed in the two receptor positive patients (cases 5 and 6 with, respectively, $1{\cdot}2$ and $2{\cdot}2 \times 10^9$ eosinophils/l before corticotherapy *versus* 0.05 and $0{\cdot}1 \times 10^9$ eosinophils/l after therapy). In contrast, persistent blood hypereosinophilia ($> 1{\cdot}5 \times 10^9$/l) was observed in the three receptor-negative patients (cases 10, 11 and 12 with,

respectively, 49·7, 8·5 and $9·1 \times 10^9$ eosinophils/l before corticotherapy *versus* 35·6, 5·4 and $4·3 \times 10^9$ eosinophils/l after therapy).

DISCUSSION

Our data indicate that eosinophils from hypereosinophilic patients are heterogeneous with regard to their glucocorticoid binding capacities. Some bear high-affinity and saturable glucocorticoid receptors with characteristics similar to those previously described in normal eosinophils (Peterson *et al.*, 1981). Others show absence or loss of detectable glucocorticoid binding in freshly purified eosinophils, when mononuclear cells or neutrophils from these patients normally bind steroid hormone under the same conditions. The threshold of detection limit in binding corresponds to 15–20% of the normal levels. In addition, binding assays do not necessarily reflect the cellular content of functional glucocorticoid receptor proteins. For this reason, we further investigated the glucocorticoid binding effect and its functional consequences.

The lack of glucocorticoid binding can be attributed to the presence of altered receptors or to the absence of glucocorticoid receptor protein. This defect was not due to metabolic alterations such as cellular ATP depletion which has been shown to result in the nuclear accumulation of null receptors, unable to bind the steroid (Wheeler *et al.*, 1981; Mendel, Bodwell & Munck, 1986). In our experimental conditions, similar cellular ATP concentrations were found in eosinophils having or lacking glucocorticoid binding sites. In the few cases studied in this respect, a correlation was observed between the presence or absence of specific binding sites and of respectively immunodetectable or undetectable receptor protein in cytosolic extracts using an anti-glucocorticoid receptor monoclonal antibody. In the glucocorticoid receptor negative samples, the immunochemical assay reveals the absence of the aminoterminal immunogenic domain of the receptor molecule, which is distinct from the carboxyterminal steroid binding domain. The failure to detect receptor protein could have been related to a proteolytic degradation of the receptor molecule. However, the cytosolic extracts were prepared from freshly highly purified viable eosinophils with a low percentage of neutrophils (2–8%) as contaminant cells. The possible role of neutrophil elastase resistant to protease inhibitors used in our study (Distelhorst & Miesfeld, 1987) is not relevant, since receptor fragments ($M_r \sim 52$ kD and 30 kD) derived from intact glucocorticoid receptor by the action of such a protease (Distelhorst *et al.*, 1987) were not found in our immunoblots negative for the detection of the 90 kD protein. All these data suggest the absence of glucocorticoid receptor protein. Different approaches, using cDNA probes, are now in progress to define the expression of glucocorticoid receptor genes at the mRNA level in cells negative for binding or immunochemical assays.

We have attempted to correlate glucocorticoid binding with *in vitro* and *in vivo* eosinophil sensitivity to glucocorticoid. The cells that did not bind glucocorticoid did not respond to dexamethasone, which inhibits the chemotaxis of normal eosinophils (Altman *et al.*, 1981). This biological response is clearly mediated by glucocorticoid receptors, since specific anti-glucocorticoids (Gagne *et al.*, 1985; Rousseau *et al.*, 1979) are able to counteract the dexamethasone effect. In one chemotaxis assay, eosinophils having binding sites did not respond to the inhibitory effects of dexamethasone. The occurrence of a steroid unresponsive state despite the presence of glucocorticoid receptors has already been reported in other cellular models (Darbre & King, 1987; Ravindran, Danielsen & Stallcup, 1987). Persistent blood hypereosinophilia ($> 1500/mm^3$) was noted in three receptor-negative patients who had received therapeutic doses of prednisone (1 mg/kg). The mechanisms by which glucocorticoid is active on the eosinophil lineage are not univocal, as shown by studies on eosinophil adherence, eosinophil migration (Altman *et al.*, 1981), eosinophil production (Butterfield *et al.*, 1986) or possible effect on T lymphocytes (Sanderson, Warren & Strath, 1985). Eosinophil sensitivity is not exclusively mediated by glucocorticoid receptors. Other means of corticoid action, such as membrane effects, have been established in other cellular models (Picart, Homo & Duval, 1980). In our study, the corticoresistant patients exhibited higher blood eosinophil counts than did corticosensitive subjects. The failure to detect functional glucocorticoid receptor could be restricted to eosinophil subpopulations. A previous report showed that some eosinophils termed hypodense eosinophils appear less sensitive to glucocorticoid than normodense eosinophils (Prin *et al.*, 1983). In the present study, most of the positive or negative binding assays were performed with cells from band IV in which a good yield of pure eosinophils is recovered. This selected cell population may not be representative of the whole eosinophil population. Further studies with blood or tissue eosinophils obtained from a large number of patients could be informative in defining whether the loss of glucocorticoid binding sites may only occur in particular eosinophil subpopulations.

In the present study HES patients having eosinophil glucocorticoid binding sites did not exhibit leukaemic markers. In one HES patient, blood eosinophils initially positive for glucocorticoid binding assays became negative at a time when the patient expressed leukaemia markers. A previous study reports that HES patients with leukaemic markers respond poorly to steroids (Parillo *et al.*, 1978). These observations are in accordance with conclusions drawn from the studies of tumour cells in leukaemia or lymphoma (Darbre & King, 1987) which can be associated with blood hypereosinophilia (Prin *et al.*, 1988). The molecular basis of the absence or loss of glucocorticoid receptors remains to be elucidated. This may reflect corticoresistant cell variants with altered receptor proteins related to genetic mechanisms occurring in clonal subpopulations. This may also be due to processing arising during cell growth or cell differentiation.

Our results emphasize a variability in the content of glucocorticoid receptor in eosinophils from different hypereosinophilic patients. No absolute correlation was found between expression of glucocorticoid recptors and eosinophil sensitivity to glucocorticoid. Nevertheless, the absence of glucocorticoid binding sites was correlated with the absence *in vitro* of glucocorticoid sensitivity and with partial *in vivo* corticoresistance, and may be useful to predict a lack of response to glucocorticoid therapy at conventional therapeutic doses. The definition of the specific target cells involved in the binding defect, the exact mechanisms modulating glucocorticoid receptor expression and the relation to a particular evolutive stage of the disease or to distinct entities among HES requires additional investigations.

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